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1 HEMP YIELDS AND ITS ROTATION EFFECTS ON WHEAT UNDER RAINFED

2 MEDITERRANEAN CONDITIONS

3

4

5 ABSTRACT

6 Hemp (*Cannabis sativa* L.) has a low impact on the environment requiring few added resources,
7 and has multiple downstream applications. There is little information on hemp biomass, seed
8 yields, and response to NPK fertilization under humid rainfed Mediterranean conditions.
9 Moreover, the effects of hemp on subsequent wheat (*Triticum aestivum* L.) crops have not been
10 determined. To address these issues, we carried out a field study for 6 years in Catalonia
11 (northeast Spain). Hemp treatments included: hemp monoculture, unfertilized hemp succeeding
12 wheat, and NPK-fertilized hemp succeeding wheat. In turn, wheat treatments included: wheat
13 monoculture, first-, second-, and third-yr wheat succeeding unfertilized hemp, and first-, second-
14 , and third-yr wheat succeeding NPK-fertilized hemp. The hemp biomass yields (5340–10,090
15 kg ha⁻¹) were similar to or lower than those achieved in other European regions whereas the
16 hemp seed yields were relatively high (604–1434 kg ha⁻¹). Both the biomass yield and seed yield
17 greatly increased with NPK fertilization. The rotation effects of hemp on the subsequent wheat
18 crops increased the wheat yield by 1368 and 155 kg ha⁻¹ in the first and second years,
19 respectively, but in the third year the yield was similar to the wheat monoculture. The beneficial
20 effects of hemp on wheat therefore appear to last for 2 years. We conclude that dual-purpose
21 hemp (harvested for fiber and seed) is an excellent predecessor for wheat, improving the
22 sustainability of cereal-based cropping systems under humid rainfed Mediterranean conditions.

23

24

25 Cropping systems in the rainfed Mediterranean region of the southern Pyrenees (northeast Spain)
26 are mainly based on cereal monocultures such as wheat (*Triticum aestivum* L.) and barley
27 (*Hordeum vulgare* L.), some break crops such as rapeseed (*Brassica napus* L.), and forage crops
28 such as Italian ryegrass (*Lolium* spp.) and alfalfa (*Medicago sativa* L.) in more humid areas
29 (Cantero-Martínez et al., 2012). The incorporation of alternative crops could improve
30 sustainability and increase the economic viability of these cropping systems (Cosentino et al.,
31 2013). One possibility is the reintroduction of fiber hemp (*Cannabis sativa* L.) as a break crop.
32 Hemp was traditionally grown in the temperate Mediterranean areas of the pre-Pyrenees, with
33 cool winters (400–900 m above sea level) and relatively humid summers (600–700 mm of annual
34 rainfall). However, hemp cultivation in these areas declined over the 1990s due to low fiber
35 prices, which led farmers to reduce the fertilizer rates applied. The European Union subsidy for
36 fiber crops (over 600 € ha⁻¹) enabled growers to continue growing hemp until 2006, when the
37 subsidy was abandoned due to the relocation of the hemp straw processing industry (Agrofibra
38 SL) (Gorchs et al., 2006).

39 Fiber hemp is a renewable source of feed and industrial products (Struik et al., 2000, Amaducci
40 et al., 2015). In Spain, hemp is mainly used as a raw material for the paper pulp industry (Gorchs
41 and Lloveras, 2003), offering a more sustainable alternative to other fiber crops (Van der Werf et
42 al., 1996). Hemp is also a promising new energy crop for conversion to bioethanol, biogas, and
43 biomass for combustion (Zegada-Lizarazu and Monti, 2011; Amaducci et al., 2015).
44 Furthermore, hemp is a dual-purpose crop because it can be harvested for both fiber and the
45 valuable, nutritious seed which is mainly produced for its high-quality oil for food (Callaway,

46 2004; Amaducci et al., 2015). Traditionally, hemp has been grown for fiber productions, and has
47 been harvested at the peak of flowering (male flowering in dioecious varieties) (Bocsa and
48 Karus, 1998), when primary bast fiber yield reaches its maximum and the proportion of lignified
49 fiber is low (Amaducci et al., 2008a). Growing hemp as a dual-purpose crop has recently gained
50 interest when fiber or biomass are intended for uses not demanding high quality fiber (pulp for
51 paper, bioenergy, etc.). On the other hand, little is known about the seed yield of hemp in
52 southern Europe, which has been proposed as a suitable region for dual-purpose hemp
53 production (Stutterheim et al., 1999). Most previous studies concerning hemp seed yields were
54 conducted in central and northern Europe (Meijer et al., 1995; Mediavilla et al., 1999; Vogl et
55 al., 2004; Maļceva et al., 2011, Faux et al., 2013, Delauran and Flengmark, 2005), in
56 Mediterranean area with irrigation (Tang et al., 2016) or Canada (Girouard et al., 1999; Vera et
57 al., 2004; Aubin et al., 2016). Moreover, residual soil nitrogen in hemp is not quantified in the
58 bibliography to date. Evaluating it in hemp under Mediterranean conditions can facilitate the
59 assessment of its response to N and help to improve nitrogen utilization, which is an important
60 agronomic, economic and environmental goal in agricultural system (Zhang et al., 2015).

61 Fiber hemp is ideal for incorporation into cereal monocultures for four major reasons. First, it is
62 seeded in spring with a short growing cycle (120–150 days), allowing subsequent seeding of the
63 winter cereal (Gorchs and Lloveras, 2003). Second, it is easy to cultivate and can be grown in a
64 wide range of environmental conditions (Gorchs and Lloveras, 2003; Zegada-Lizarazu et al.,
65 2010). Third, it can contribute to control diseases, pests and particularly weeds (hemp has an
66 extremely vigorous growth after emergence, which rapidly smothers weeds and allows the
67 reduction of herbicide applications to the subsequent cereal crop) (Struik et al., 2000; Van der
68 Werf et al., 1996; Zegada-Lizarazu and Monti, 2011). Finally, hemp improves the soil structure

69 due to its deep root system and the large quantity of organic residues left on the ground
70 (Amaducci et al., 2008b). It is well known that crop rotation increases yields of the subsequent
71 crops compared to monocultures (Bullock, 1992). Nevertheless, the rotation effects of hemp on
72 subsequent crops have not been studied in detail because of the low acreage grown with hemp
73 (Zegada-Lizarazu and Monti, 2011). Farmers growing wheat after hemp have reported yield
74 increases of 10–20% compared to wheat monoculture (Bocsa and Karus, 1998; Gorchs and
75 Lloveras, 1998). The careful analysis of hemp seed yields and the rotation effects on subsequent
76 crops is necessary to evaluate the adaptability of this crop and its potential inclusion in rainfed
77 Mediterranean cropping systems. We therefore set out a 6-yr study to evaluate hemp biomass
78 and seed yields under rainfed Mediterranean conditions, and to quantify the rotation effects of
79 hemp on the subsequent wheat crops and the soil nitrate content.

80

81

MATERIALS AND METHODS

Field location, soil and weather characteristics

83 A field trial was conducted over 6 consecutive years (1994–1995 to 1998–1999) in a commercial
84 field in the pre-Pyrenean area of Merlès (northeast Spain, 525 m above sea level). The soil was
85 classified as a Typic Eutrudept (Soil Survey Staff, 2003) with a sandy-loam texture, and had the
86 following characteristics at the beginning of the experiment (0–30 cm): pH = 8.2; organic matter
87 (Walkley and Black, 1934) = 17 g kg⁻¹; P (Olsen method) = 17 mg kg⁻¹; and K = 211 mg kg⁻¹
88 (ammonium acetate method). The experiment was conducted under rainfed Mediterranean
89 conditions. A weather station located in the field (Campbell Scientific Ltd, Leicestershire, UK)
90 recorded air temperature, solar radiation and rainfall. Weather conditions are reported in Table 1.

91

92 ***Experimental design and treatments***

93 The experiment was arranged in a randomized complete block design with four replications. The
94 experimental field area was 80 m x 32 m (2560 m²) and was seeded with sunflower (*Helianthus*
95 *annuus* L.) in 1992–1993 and with wheat in 1993–1994. In 1994–1995, 32 plots were established
96 following eight crop rotation schemes (Table 2) randomly assigned within each block. A plot
97 was 10 m x 5 m with 2 m of border between plots, which were sown with either wheat or hemp,
98 correspondingly. Outside the trial, the rest of the field was sown with wheat.

99 ***Hemp treatments.*** Hemp was grown in different plots from 1994-95 to 1996-97 (Table 2). Hemp
100 treatments included: i) unfertilized hemp after wheat (UH), ii) fertilized hemp after wheat (FH),
101 and iii) fertilized hemp monoculture (FHM) (Tables 2 and 3). Fertilized hemp received 100, 35
102 and 130 kg ha⁻¹ yr⁻¹ of N [ammonium nitrate (NH₄NO₃), 33.5% N], P [monocalcium phosphate
103 (Ca(H₂PO₄)₂, 16 % P₂O₅) and K [potassium chloride (CLK), 60 % K₂O], respectively. Note that
104 treatment FHM was only present in 1995-96 and 1996-97 (Table 2). This design allowed to
105 evaluate the effects of NPK fertilization on hemp production by comparing UH with FH in 1995,
106 1996 and 1997, as well as the effect of the preceding crop (wheat or hemp) on hemp production
107 by comparing FH with FHM in 1996 and 1997 (Tables 2 and 3).

108 ***Wheat treatments.*** Wheat was grown from 1994-95 to 1998-99. Wheat treatments included i)
109 first-yr wheat following unfertilized hemp (UH1W), ii) first-yr wheat following fertilized hemp
110 (FH1W), iii) second-yr wheat following unfertilized hemp (UH2W), iv) second-yr wheat
111 following fertilized hemp (FH2W), v) third-yr wheat following unfertilized hemp (UH3W), vi)
112 third-yr wheat following fertilized hemp (FH3W), and vii) wheat monoculture (WM) (Tables 2
113 and 3). Note that these treatments were not present each year of the study. This design allowed to

114 evaluate the rotation effects of hemp on the three subsequent wheat crops by comparing WM
115 plots against (i) UH1W and FH1W plots in 1996, 1997 and 1998; (ii) UH2W and FH2W plots in
116 1997, 1998 and 1999; and (iii) UH3W and FH3W plots in 1998 and 1999 (Table 3).

117

118 *Crop management and measurements*

119 ***Hemp management.*** The soil was plowed with a chisel, disk and a cultivator with a roller. In
120 fertilized hemp treatments, the fertilizer was incorporated into the soil before seeding, whereas
121 unfertilized hemp received no NPK fertilization. Hemp was seeded between Apr. 20 and May 5,
122 depending on the year, by using an experimental plot seeder that includes eight rows with 15 cm
123 between rows. Seeding was carried out after rainfall (to guarantee optimal emergence) with a
124 small tractor to facilitate the seeding of hemp plots without damaging wheat plots. The typical
125 variety grown by farmers in the region ('Futura 77') was seeded at 350 seeds m⁻² (with over 90%
126 viable seeds). This hemp variety is intended for fiber production, has a medium growing cycle, is
127 monoecious (each plant bears both male and female flowers), and is maintained by Fédération
128 Nationale des Producteurs de Semences de Chanvre (Mediavilla et al., 1999). No herbicides were
129 used, as weeds were adequately suppressed by the crop. Similarly, no phytosanitary treatments
130 were applied during the growing cycle, as is usual practice among hemp farmers in Spain
131 (Gorchs and Lloveras, 2003). Hemp was sampled twice: i) for biomass at flowering (2302–2304
132 stage, Mediavilla et al., 1998), when fiber content and fiber quality are expected to be high, and
133 ii) for biomass and seed yield (dual purpose) at seed maturity (2306–2307 stage, Mediavilla et
134 al., 1998). The flowering sampling took place between Aug. 4 and 7 and the seed-maturity
135 sampling took place between Sept. 13 and 19. In 1996, hemp could not be harvested for seed
136 because the crop was severely damaged by a violent hail storm which occurred just after

137 flowering sampling (Mediavilla stage 2304). About half of the stalks were broken and felt to the
138 ground along with all leaves and inflorescences. Such severe crop damage influenced the farmer
139 to harvest the whole field. After cutting and removing the hemp border with a bar mower,
140 aboveground hemp biomass was determined by taking manually two subsamples of 0.6 m² (1.2
141 m²) per plot (including four hemp rows 1 m long x 0.15 m within rows per subsample). Hemp
142 plants in each subsample were cut at ground level with a bar mower, the number of plants was
143 counted, and the fresh biomass was weighed. The moisture content was determined by taking
144 and chopping 300 g of plant material from each subsample and drying at 105°C for 24 h. The
145 stem yield was determined by randomly selecting 5 plants per subsample (10 plants per plot),
146 separating the stem from the leaves and the inflorescences, drying the three plant fractions at
147 105°C for 24 h, and weighting them. Afterwards, the inflorescences were left about 15 days on
148 trays in the laboratory and then the seed yield was determined by threshing the 10 inflorescences
149 per plot using a Walter-Wintersteiger LD 180 (Ried, Austria) and drying the seeds at 105 °C for
150 24 h. After picking the samples, the remaining biomass was harvested by the farmer and sold for
151 industrial purposes.

152 ***Wheat management.*** The soil was plowed with a chisel, disk and a cultivator with a roller.
153 Winter wheat was seeded with a cereal drill between Oct. 25 and Nov. 3, depending on the year,
154 at 420 seeds m⁻² (viable seeds over 92%) with 15 cm between rows. Cv. ‘Soissons’ hard red
155 winter type, medium-late to maturity, was chosen due to its good adaption to Mediterranean
156 areas with relatively cold winters (Serra et al., 2012). Wheat was fertilized with 120, 35, and 110
157 kg ha⁻¹ yr⁻¹ of N [ammonium nitrate (NH₄NO₃), 33.5% N], P [monocalcium phosphate
158 (Ca(H₂PO₄)₂, 16 % P₂O₅], and K [potassium chloride (CLK), 60 % K₂O], respectively. Nitrogen
159 fertilizer was divided into two equal applications at Zadocks stages 21–23 (tillering) and 30

160 (stem elongation) (Zadocks et al., 1974). Weeds were controlled by post-applying at Zadocks
161 stages 23-29 (tillering) 2.5 l ha⁻¹ of a mixture of 7.5% Ioxynil (4-hydroxy-3,5-
162 diiodobenzonitrile), 7.5% Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and 37.5% MCPP
163 (2-(4-chloro-2-methylphenoxy) propionic acid). Tralkoxidim (2-(1-(Ethoxyimino)propyl)-3-
164 hydroxy-5-(2,4,6-trimethylphenyl)cyclohex-2-enone) was used to control wild oat (*Avena sterilis*
165 *L. subsp. ludoviciana* and *A. sterilis*, subsp. *sterelis*) when needed (375 g ha⁻¹ a.i) at Zadocks
166 stages 31-32 (stem elongation) . Wheat was harvested by hand between June 27 and July 12 by
167 taking four subsamples of 0.9 m² (3.6 m²) per plot with a hedge trimmer, and all aboveground
168 biomass was put in a bag. In the laboratory, subsamples were weighed, the plants were threshed,
169 and the seed was weighed. Stover was dried in the oven at 105°C for 24 h before calculating the
170 aboveground dry matter weight (biomass). The seed moisture content was determined from a
171 300-g sample using a Dickey John moisture tester (Dickey John Corp, Auburn, IL, USA). The
172 seed yield was adjusted to 14% moisture.

173

174 ***Soil nitrate content***

175 The soil nitrate content was determined each year after harvest at seed maturity in hemp plots,
176 and at tillering and after harvest in wheat plots. Soil samples were taken to a depth of 90 cm in
177 30-cm increments using Eijkelkamp cylindrical augers (four cores per plot by each layer).
178 Nitrates were extracted using deionized water (1:1 water to 100 g fresh soil ratio) and were
179 measured using two Merckoquant test strips (Merck, Darmstadt, Germany) with a Nitrachek®
180 device (Eijkelkamp, Giesbeek, Netherlands) calibrated according the standard procedure
181 (Bischoff et al., 1996). Soil moisture content was determined by taking 5 g per subsample and

182 drying it at 105°C for 24 h. Nitrate concentrations were converted into $\text{kg NO}_3\text{-N ha}^{-1}$ by using
183 the average apparent density from 1996 to 1998, i.e. 1430 kg m^{-3} .

184

185 *Calculations*

186 The rotation effects of hemp on the succeeding three wheat crops were based on wheat grain
187 yields and determined as follows: i) First-yr wheat (1996, 1997, and 1998): (UH1W – WM) for
188 UH1W, and (FH1W – WM) for FH1W; ii) Second-yr wheat (1997, 1998, and 1999): (UH2W –
189 WM) for UH2W, and (FH2W – WM) for FH2W; iii) Third-yr wheat (1998 and 1999): (UH3W –
190 WM) for UH3W, and (FH3W – WM) for FH3W.

191 The rotation effects were expressed in kg ha^{-1} of grain at 14% moisture content.

192

193 *Statistical analysis*

194 Data representing the variables from every individual year and from all years together were
195 processed by analysis of variance (ANOVA) using proc GLM, SAS v8.0 (SAS Institute, 1999).
196 The treatments included in each analysis are shown in Table 3. Means were compared by using
197 the protected least significant difference (LSD) ($P < 0.05$). In addition, we used a-priori
198 orthogonal contrasts to test the impact of crop rotation on wheat yields and biomass. We grouped
199 “UH1W and FH1W” versus “WM” for the effect of crop precedent on first-yr wheat, “UH2W
200 and FH2W” versus “WM” for the effect of crop precedent on second-yr wheat, and “UH3W and
201 FH3W” versus “WM” for the effect of crop precedent on third-yr wheat.

202

203

RESULTS AND DISCUSSION

204 ***Hemp characterization***

205 ***Hemp biomass and stem yield.*** At flowering, hemp biomass ranged from 5311 to 10,983 kg ha⁻¹
206 and the stem yield ranged from 3213 to 8061 kg ha⁻¹, depending on the year and fertilization
207 treatment (Table 4). At seed maturity, hemp biomass ranged from 4737 to 9393 kg ha⁻¹, and stem
208 yield varied between 3163 and 6386 kg ha⁻¹. These biomasses tended to be lower than those
209 reported for northern Italy (Amaducci et al., 2002), southern Italy (Cosentino et al., 2013), and
210 the Netherlands (Struik et al., 2000), probably because of the milder summer temperatures, and a
211 more even rainfall distribution in northern European regions, and due to the irrigation in southern
212 Italy.

213 On average across treatments, hemp biomass increased by 1182 kg ha⁻¹ between flowering and
214 seed maturity in 1995, but decreased by 911 kg ha⁻¹ in 1997 (data derived from Table 4).

215 Hemp can lose large amounts of dry matter during the growing season (up to 3000 kg ha⁻¹)
216 because leaves rapidly shed and many plants die as a result of self-thinning (Van der Werf et al.,
217 1996), particularly under water-stress conditions such as those observed in August 1997 in our
218 study (Table 1).

219 ***Hemp seed yield.*** The seed yield under rainfed Mediterranean conditions averaged 1044 kg ha⁻¹
220 across years and fertilization treatments (Table 4). The seed yields obtained in this study were
221 similar to or higher than the 300–1100 kg ha⁻¹ range reported in many studies conducted in
222 central and northern Europe (Meijer et al., 1995; Mediavilla et al., 1999; Vogl et al., 2004;
223 Delauran and Flengmark, 2005), although other studies in northern Europe achieved similar or
224 higher seed yields (Callaway, 2004; Małceva et al., 2011; and Faux et al., 2013). The relatively
225 high hemp seed yields achieved in our study seems to confirm the suitability of relatively humid
226 and rainfed Mediterranean environments for dual-purpose hemp production (stems + seeds),

227 especially if the hemp fibers are used for paper pulp, because this industry does not require high-
228 quality fibers.

229 ***Fertilization of hemp with NPK.*** Fertilization of hemp with NPK significantly increased hemp
230 biomass and stem yields in 1996 and 1997, but not in 1995 (Table 4). The lack of response to
231 NPK fertilization in 1995 may be due to sufficient soil P (17 mg kg⁻¹) and K (211 mg kg⁻¹) levels
232 (Villar and Aran, 2008), and possibly due to a high soil N content at sowing (data not available),
233 given that winter and spring were dry in 1995 (71 mm from Dec. to Apr.) but wet in 1996 (412
234 mm) and in 1997 (240 mm).

235 ***Effect of crop rotation on hemp production.*** The preceding crop (wheat or hemp) did not
236 significantly affect hemp biomass, stem, or seed yields (Table 5), confirming that hemp performs
237 well when grown in monoculture (Bocsa and Karus, 1998). However, diseases and pests can
238 increase and fiber yields can decrease when hemp is grown in monoculture (Li, 1982). Bocsa and
239 Karus (1998) reported that second-year and third-year hemp in monoculture requires increasing
240 rates of fertilizer application. Our data suggest this may not always be the case, because our
241 second-year and third-year hemp grown in monoculture had slightly higher yields than the first-
242 year hemp after wheat at the same level of fertilizer.

243 ***Soil nitrate content after hemp harvest.*** In each individual year, the NPK fertilization of hemp
244 did not significantly change the soil NO₃-N content after harvest (compared to UH) except for
245 the 30–60 cm layer in 1996 (Figure 1). The application of 100 kg N ha⁻¹ only increased the soil
246 NO₃-N content (0–90 cm) by an average of 7 kg ha⁻¹ (33 kg N ha⁻¹ for UH and 40 kg N ha⁻¹ for
247 FH), thus reflecting a high nitrogen use efficiency. The preceding crop (wheat or hemp) did not
248 affect soil NO₃-N levels at any depth after the hemp harvest in any year (Figure 1). Over 2 years,
249 the soil NO₃-N content (0–90 cm) after the hemp seed harvest averaged 42 kg N ha⁻¹ in the FHM

250 plots and 46 kg N ha⁻¹ in the FH plots. The low soil nitrate levels observed in the FH plots after
251 hemp harvest together with the brief period between hemp harvest and wheat sowing (1–2
252 months at the most) suggest a low N leaching risk for hemp production under rainfed
253 Mediterranean conditions.

254

255 ***Wheat characterization***

256 ***Wheat yields.*** Across wheat treatments, the average wheat grain yields were 4832, 2408, 3690
257 and 3063 kg ha⁻¹ in 1996, 1997, 1998 and 1999, respectively (data derived from Table 6). Over
258 the last three years, yields in our study were lower than the long-term average yields for the
259 region (4000–5000 kg ha⁻¹) (Cantero-Martínez et al., 2012), probably reflecting the low rainfall
260 levels in 1998 and 1999 (Table 1) as well as the high average temperatures in May (13.6°C in
261 1996, 15.4°C in 1997, 14.2°C in 1998, and 16.1°C in 1999), compared to the average of 12.8°C
262 from 1951 to 1990. Additionally, low wheat yields in 1997 could be attributed to an extended dry
263 period from February to May 20 (-174 mm below the 40-yr average) that reduced the number of
264 ears (- 20 %) as well as the number of grains per ear (-33 %) (data not presented).

265 ***Effect of hemp rotation on the subsequent wheat crops.*** Plots where first-yr wheat was
266 preceded by hemp (UH1W and FH1W) had higher grain yields and biomass than WM plots in
267 1996, 1997 and 1998 (Table 6). The rotation effects of hemp on first-year wheat averaged 1368
268 kg ha⁻¹ for grain (+47%) (Table 7) and 2283 kg ha⁻¹ (+38%) for biomass (data not reported).
269 Plots where second-yr wheat was preceded by hemp (FH2W and UH2W) had significantly
270 higher grain yields and biomass than WM plots in 1997 and 1998, but had similar grain and
271 biomass yields to WM in 1999 (Table 6). The rotation effects of hemp on second-year wheat
272 averaged 155 kg ha⁻¹ (+6%) for grain (Table 7) and 431 kg ha⁻¹ (+8%) for biomass (data not

273 reported). Finally, the grain and biomass yields of third-year wheat succeeding hemp (UH3W
274 and FH3W) were similar to those of WM in both 1998 and 1999 (Table 6), suggesting the
275 positive effect of hemp rotation on subsequent wheat crops lasts a maximum of 2 years.
276 This is the first study trying to quantify the rotation effects of hemp on subsequent crops. The
277 rotation effects of hemp on wheat observed in this study are higher than the +10 to 20%
278 estimated by farmers (Bocsa and Karus, 1998, Gorchs and Lloveras, 1998), and also higher than
279 those reported for other crop rotations involving wheat: (i) 982 kg ha⁻¹ (+46%) for wheat
280 succeeding faba beans (*Vicia faba* L.) compared to WM under rainfed Mediterranean conditions
281 in Spain (López-Bellido et al., 1996); (ii) 400 to 600 kg ha⁻¹ (+15%) for wheat after canola
282 (*Brassica napus* L.), field pea (*Pisum sativum* L.), and fallow compared to WM in a northern
283 semi-arid climate in Canada (Arshad et al., 1998); and (iii) 500 kg ha⁻¹ for wheat rotated with
284 grain sorghum (*Sorghum bicolor* L.) or fall canola against WM in Texas, USA (Unger, 2001).
285 Our results confirm the opinion of the farmers that hemp is an excellent predecessor for wheat
286 under rainfed Mediterranean conditions.

287

288 ***Soil nitrates at wheat tillering and after wheat harvest***

289 Mean residual soil NO₃-N (0-90 cm) over the 3-year study was 39 kg ha⁻¹ at wheat tillering, and
290 54 kg ha⁻¹ at wheat harvest (Figure 2), ranging across years from 27 to 54 kg NO₃-N ha⁻¹ at
291 tillering and from 40 to 72 kg NO₃-N ha⁻¹ at harvest (data derived from Figure 2). At wheat
292 harvest, residual soil nitrate content at 0-30 cm depth was 32, 36, and 52 kg NO₃-N ha⁻¹, for
293 1996, 1997 and 1998, respectively. In 1998, at wheat harvest, high residual soil nitrate content at
294 0-30 cm might be attributed to water stress (Table 1). Indeed, in Mediterranean areas, water
295 deficits have been reported to decrease N uptake and reduce wheat grain yield (López Bellido et

296 al., 2005). Furthermore, high N content in hemp leaves (4 to 5% N) (Iványi and Izsáki, 2009)
297 might have contributed to increased soil nitrate content in wheat succeeding hemp plots as a
298 result of leaf shedding and mineralization. In addition, in Mediterranean areas, some residual
299 nitrogen may remain as nitrate in dry years (López Bellido et al., 2005).

300 In first-year wheat, crop rotation did not affect soil nitrate content (0–90 cm) at tillering or at
301 harvest (Figure 2).

302 Soil nitrate content at 0–30 cm depth in plots where hemp was previously grown was comparable
303 to WM. Contrastingly, in deep soil layers (30–90 cm), plots where hemp was previously grown
304 tended to have lower nitrate concentrations than WM (52% lower on average), which is
305 consistent with the deep root system of hemp (Amaducci et al., 2008b). These results suggest
306 that hemp could also play a role as a catch crop for nitrates, which is important in nitrate-
307 vulnerable zones.

308

309

CONCLUSIONS

310 The hemp biomass and seed yields in our field study ranged from 5340 to 10,090 kg ha⁻¹ and
311 from 604 to 1434 kg ha⁻¹, respectively, regardless of the preceding crop (hemp or wheat).
312 Fertilization of hemp with NPK significantly increased hemp biomass and stem yield without
313 significantly increasing the soil NO₃-N content after hemp harvest, reflecting a high fertilizer use
314 efficiency. Compared to wheat monoculture, the inclusion of hemp in the crop rotation increased
315 first-year wheat yields by an average of 1368 kg ha⁻¹ (+47%) and second-year wheat yields by an
316 average of 155 kg ha⁻¹ (+6%), but there was no yield increase in third-year wheat yields. These
317 results suggest that the positive effect of hemp on the subsequent wheat crops lasts a maximum

318 of 2 years. Similarly, plots where hemp was grown tended to have lower soil nitrate contents in
319 the deep soil layers than wheat monoculture plots. This study has revealed the quantitative
320 effects of hemp rotation on subsequent wheat crops, and confirms that dual-purpose hemp has
321 the potential to improve the long-term sustainability of cereal-based cropping systems under
322 rainfed Mediterranean conditions in southern Europe.

323

324

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441

FIGURE CAPTIONS

442

443 **Figure 1.** Soil NO₃-N content (kg N ha⁻¹) after hemp seed harvest, for two fertilizer treatments
444 (upper three figures) and for two crop rotations (lower two figures). Horizontal lines depict least
445 significant differences ($P < 0.05$) at each depth when significant differences exists. Numbers
446 within plots indicate total soil NO₃-N content (kg N ha⁻¹) from 0 to 90 cm for each treatment. ns -
447 not significant.

448

449 **Figure 2.** Soil NO₃-N (kg N ha⁻¹) at wheat tillering (upper figures) and after wheat harvest
450 (lower figures), for three crop rotation treatments. Horizontal lines depict least significant
451 differences ($P < 0.05$) at each depth when significant differences exist. Numbers within plots
452 indicate total soil NO₃-N content (kg N ha⁻¹) from 0 to 90 cm for each treatment. ns - not
453 significant.

454

455

TABLES

456 **Table 1.** Rainfall totals and average air temperatures at Merlès (Barcelona province) during each
 457 year of the field test, plus the mean historic values (1951–1990).

	Rainfall (mm)						Temperature (°C)					
	94-95	95-96	96-97	97-98	98-99	Mean†	94-95	95-96	96-97	97-98	98-99	Mean†
Year	567	969	729	469	555	695	13.2	12.4	12.7	12.5	11.8	11.4
Wheat‡	158	560	388	320	283	336	9.5	8.5	8.7	7.7	6.7	7.2
Hemp1§	147	322	204	73	179	229	18.7	17.6	18.2	18.6	-	16.6
Hemp2¶	346	397	280	156	203	311	19.2	18.4	19.1	19.4	-	17.4

458 † Mean values 1951–1990 in Prats del Lluçanès, located 4 km northeast of Merlès (Barcelona province,
 459 Spain).

460 ‡ Wheat = Nov. through May

461 § Hemp 1 = May through July (harvest at flowering).

462 ¶ Hemp 2 = May through Aug. (harvest for biomass and seed)

463

464 **Table 2.** Basic scheme of crop rotation studied for hemp (darker cells) and wheat (clearer cells)
 465 treatments and years when each treatment was present.

	Wheat Monoculture †	Hemp-Wheat rotation ‡						Hemp Monoculture §
Year	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
1994-95	<i>W</i>	UH	FH	<i>W</i>	<i>W</i>	<i>W</i>	<i>W</i>	<i>FH</i>
1995-96	WM	UH1W	FH1W	UH	FH	<i>W</i>	<i>W</i>	FHM
1996-97	WM	UH2W	FH2W	UH1W	FH1W	UH	FH	FHM
1997-98	WM	UH3W	FH3W	UH2W	FH2W	UH1W	FH1W	
1998-99	WM			UH3W	FH3W	UH2W	FH2W	

466 † *W* = wheat (not included in the comparisons). WM = wheat monoculture.

467 ‡ UH = unfertilized hemp succeeding wheat. FH = fertilized hemp (100 kg N ha⁻¹, 35 kg P ha⁻¹,
 468 and 130 kg K ha⁻¹) succeeding wheat. 1W, 2W, and 3W = first, second, and third year of wheat
 469 following UH or FH.

470 § *FH* = fertilized hemp (not included in the comparisons). FHM = fertilized hemp monoculture

471

472

473 **Table 3.** List of hemp and wheat treatments, years when each treatment was present in the study, and treatments used in each statistical
 474 analysis.

Hemp treatments	Acronym	Years grown					Treatments used in ANOVA	
		1995	1996	1997	1998	1999	Fertilization	Rotation
Unfertilized hemp	UH	X	X	X			X	
Fertilized hemp	FH	X	X	X			X	X
Fertilized hemp monoculture	FHM		X	X				X
Hemp treatments in a given year or in ANOVA		2	3	3			2	2

Wheat treatments	Acronym	Years grown					Treatments used in ANOVA		
		1995	1996	1997	1998	1999	First-yr (1W)	Second-yr (2W)	Third-yr (3W)
Wheat monoculture	WM		X	X	X	X	X	X	X
First-yr wheat succeeding UH	UH1W		X	X	X		X		
First-yr wheat succeeding FH	FH1W		X	X	X		X		
Second-yr wheat succeeding UH	UH2W			X	X	X		X	
Second-yr wheat succeeding FH	FH2W			X	X	X		X	
Thid-yr wheat succeeding UH	UH3W				X	X			X
Third-yr wheat succeeding FH	FH3W				X	X			X
Wheat treatments in a given year or in ANOVA		0	3	5	7	5	3	3	3

475

476 **Table 4.** Hemp biomass and stem yield at flowering, and biomass, stem, and seed yield at seed maturity, for two fertilizer treatments
 477 over three years. Hemp was grown after wheat.

Fertilizer Treatment [§]	Flowering [†]								Seed maturity [‡]								
	Biomass				Stem				Biomass			Stem			Seed		
	1995	1996	1997	MEAN	1995	1996	1997	MEAN	1995	1997	MEAN	1995	1997	MEAN	1995	1997	MEAN
kg dry mass (DM) ha ⁻¹																	
UH	7655	5311	5340	6102	5440	3213	3848	4167	8886	4737	6811	5754	3163	4445	1063	604	833
FH	8260	10,983	10,090	9777	6265	8061	7809	7378	9393	8871	9132	5902	6386	6144	1434	1075	1254
MEAN	7958	8147	7715	7940	5853	5637	5829	5773	9140	6804	7972	5828	4775	5295	1434	1075	1044
Analysis of variance <i>P</i> > <i>F</i>																	
Fertil. (F)	0.490	<0.001	<0.001	<0.001	0.552	<0.001	0.014	<0.001	0.654	<0.001	0.001	0.920	0.002	0.039	0.269	0.105	0.049
Year (Y)	-	-	-	0.684	-	-	-	0.892	-	-	0.001	-	-	0.163	-	-	0.054
F x Y	-	-	-	<0.001	-	-	-	0.002	-	-	0.007	-	-	0.055	-	-	0.779
LSD ¶	-	1398	1457	815	-	934	2425	855	-	620	1267	-	1048	1589	-	-	456

478 [†] 2302–2304 stage (Mediavilla et al., 1998). [‡] 2306–2307 stage (Mediavilla et al., 1998). Hemp could not be harvested at seed

479 maturity in 1996 because a hail storm damaged the crop.

480 [§] UH = Unfertilized hemp. FH = Fertilized hemp (100 kg N ha⁻¹, 35 kg P ha⁻¹, and 130 kg K ha⁻¹).

481 ¶ Least significant difference (LSD) at *P* ≤ 0.05.

482

483 **Table 5.** Hemp biomass and stem yield at flowering, and biomass, stem yield, and seed yield at
 484 seed maturity, for two rotations over two years: fertilized hemp monoculture (FHM) and
 485 fertilized hemp after wheat (FH).

Crop rotation [§]	Flowering [†]						Seed maturity [‡]		
	Biomass			Stem			Biomass	Stem	Seed
	1996	1997	MEAN	1996	1997	MEAN	1997		
	kg dry mass (DM) ha ⁻¹								
FHM	11,236	10,268	10,751	8318	7916	8117	9135	6699	1192
FH	10,983	10,090	10,536	8061	7809	7935	8871	6386	1075
MEAN	11,110	10,179	10,644	8190	7863	8026	9003	6543	1134
	Analysis of variance $P > F$								
Rotation (R)	0.713	0.847	0.704	0.560	0.927	0.761	0.674	0.474	0.528
Year (Y)	-	-	0.111	-	-	0.589	-	-	-
R x Y	-	-	0.9470	-	-	0.899	-	-	-
LSD [¶]	-	-	-	-	-	-	-	-	-

486 † 2302-2304 stage (Mediavilla et al., 1998). ‡ 2306-2307 stage (Mediavilla et al., 1998). Hemp
 487 could not be harvested at seed maturity in 1996 because a hailstorm damaged the crop.

488 § FHM = fertilized hemp monoculture. FH = fertilized hemp succeeding wheat. In both rotations,
 489 hemp received 100 kg N ha⁻¹, 35 kg P ha⁻¹, and 130 kg K ha⁻¹.

490 ¶ Least significant difference (LSD) at $P \leq 0.05$.

491

492

493 **Table 6.**Wheat grain yield (14% moisture content) and dry biomass yield as a function of the
 494 crop rotation: wheat monoculture (WM), first-yr wheat (1W), second-yr wheat (2W), and third-
 495 yr wheat (3W) succeeding unfertilized hemp (UH) and fertilized hemp (FH). Fertilized hemp
 496 received 100 kg N ha⁻¹, 35 kg P ha⁻¹, and 130 kg K ha⁻¹.

Rotation†	Grain (kg ha ⁻¹)					Biomass (kg DM ha ⁻¹)				
	1996	1997	1998	1999	MEAN	1996	1997	1998	1999	MEAN
First-year wheat (1W)‡										
WM	3744b	1683b	3337b	-	2921c	8436c	2933b	6770b	-	6046c
UH1W	5209a	3072a	4211a	-	4164b	10,777b	5185a	8305a	-	8089b
FH1W	5544a	3262a	4437a	-	4414a	11,685a	5522a	8499a	-	8569a
Analysis of variance <i>P</i> > <i>F</i>										
Rotation (R)	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001	0.002	-	<0.001
Year (Y)	-	-	-	-	<0.001	-	-	-	-	<0.001
Y x R	-	-	-	-	0.095	-	-	-	-	0.089
LSD (Rotation)§	426	391	387	-	226	866	663	807	-	440
Contrast ¶	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001	-	<0.001
Second-year wheat (2W)										
WM	-	1683b	3337b	3164	2728b	-	2933b	6770b	6752	5485b
UH2W	-	1873b	3910a	2992	2924a	-	3169ab	8149a	6587	5968a
FH2W	-	2148a	3651ab	2726	2841ab	-	3599a	7809a	6185	5864a
Analysis of variance <i>P</i> > <i>F</i>										
Rotation (R)	-	0.006	0.009	0.059	0.115	-	0.017	0.001	0.170	0.010
Year (Y)	-	-	-	-	<0.001	-	-	-	-	0.001
Y x R	-	-	-	-	<0.001	-	-	-	-	<0.001
LSD (Rotation)	-	275	353	-	186	-	449	655	-	325
Contrast	-	0.009	0.006	0.055	0.059	-	0.025	<0.001	0.170	0.003
Third-year wheat (3W)										
WM	-	-	3337	3164	3251	-	-	6770	6752	6761
UH3W	-	-	3000	3263	3232	-	-	6080	7120	6600
FH3W	-	-	3284	3170	3227	-	-	6751	6930	6840
Analysis of variance <i>P</i> > <i>F</i>										
Rotation (R)	-	-	0.112	0.825	0.589	-	-	0.100	0.550	0.603

Year (Y)	-	-	-	-	0.964	-	-	-	-	0.390
Y x R	-	-	-	-	0.162	-	-	-	-	0.078
LSD (Rotation)	-	-	-	-	-	-	-	-	-	-
Contrast	-	-	0.187	0.736	0.501	-	-	0.254	0.352	0.847

497 † Fertilized hemp received 100 kg N ha⁻¹, 35 kg P ha⁻¹, and 130 kg K ha⁻¹.

498 ‡ Means within a column followed by the same letter are not significantly different, according to
 499 an F-protected least significant difference (LSD) ($P \leq 0.05$).

500 § Least significant difference (LSD) at $P \leq 0.05$.

501 ¶ Contrasts: wheat monoculture (WM) vs wheat succeeding fertilized and unfertilized hemp.

502

503

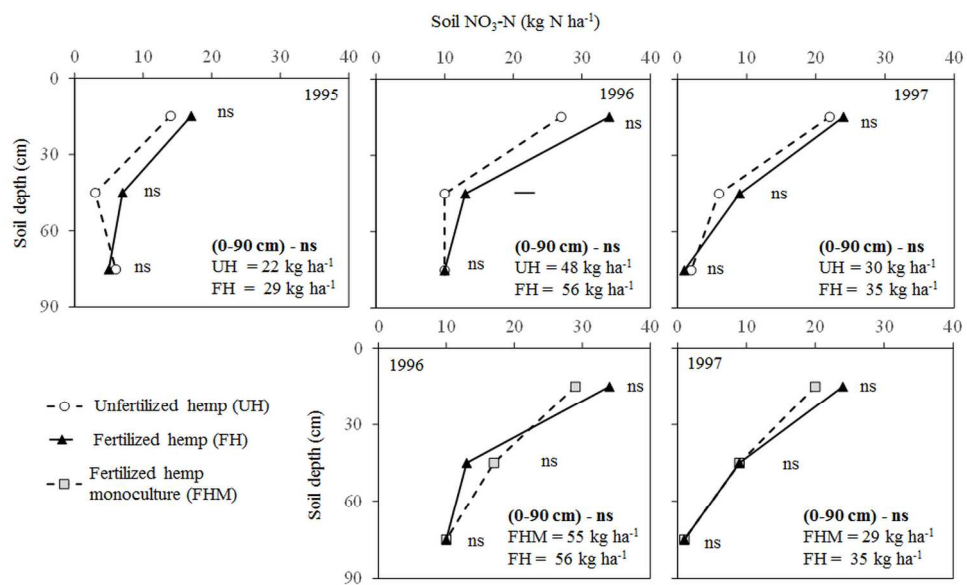
504 **Table 7.** Yield differences (rotation effects) of first-yr wheat (1W), second-yr wheat (2W), and third-yr wheat (3W) succeeding
 505 unfertilized hemp (UH) and fertilized hemp (FH) compared to wheat monoculture (WM).

Rotation effect (grain yield, kg ha ⁻¹ at 14% moisture content)													
Rotation†	1996	1997	1998	MEAN	Rotation	1997	1998	1999	MEAN	Rotation	1998	1999	MEAN
	First-year wheat (1W)					Second-year wheat (2W)					Third-year wheat (3W)		
UH1W	1465	1389	874	1243	UH2W	190	573	-172	197	UH3W	-337	99	-119
FH1W	1800	1579	1100	1493	FH2W	465	314	-438	114	FH3W	-53	6	-24
Mean	1633	1484	987	1368	Mean	328	444	-305	155	Mean	-195	53	-71
Analysis of variance <i>P</i> > <i>F</i>													
Rotation (R)	0.179	0.334	0.423	0.062		0.234	0.545	0.378	0.628		0.341	0.625	0.554
Year (Y)	-	-	-	0.417		-	-	-	0.026		-	-	0.474
Y x R	-	-	-	0.874		-	-	-	0.351		-	-	0.262
LSD (Rotation) ‡	-	-	-	-		-	-	-	-		-	-	-

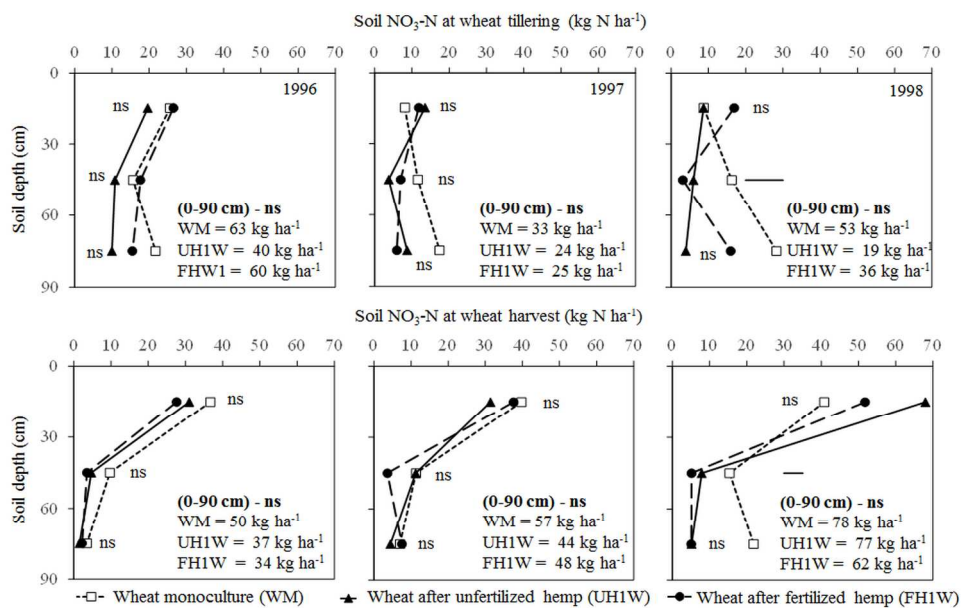
506 † Fertilized hemp received 100 kg N ha⁻¹, 35 kg P ha⁻¹, and 130 kg K ha⁻¹.

507 ‡ Least significant difference (LSD) at *P* ≤ 0.05.

508



779x480mm (96 x 96 DPI)



829x527mm (96 x 96 DPI)